Chem. Senses 30: 497–504, 2005

doi:10.1093/chemse/bji042
Advance Access publication June 8, 2005

Biochemical and Physiological Evidence that Calmodulin Is Involved in the Taste Response of the Sugar Receptor Cells of the Blowfly, *Phormia regina*

Keiji Seno[1], Tadashi Nakamura[2] and Mamiko Ozaki[1]

[1]Department of Applied Biology, Faculty of Textile Science, Kyoto Institute of Technology, Kyoto, Japan and [2]Department of Applied Physics and Chemistry, The University of Electro-Communications, Tokyo, Japan

Correspondence to be sent to: Mamiko Ozaki, Department of Applied Biology, Faculty of Textile Science, Kyoto Institute of Technology, Kyoto, Japan. e-mail: mamiko@ipc.kit.ac.jp

Abstract

The gustatory system is essential for almost all animals. However, the signal transduction mechanisms have not yet been fully elucidated. We isolated labellar chemosensilla from blowfly, *Phormia regina*, and purified calcium binding proteins from the water soluble fraction. The most abundant calcium-binding protein was calmodulin. To investigate the role of calmodulin in taste transduction, electrophysiological responses were recorded with the calmodulin inhibitor, W-7. When we stimulated the labellar chemosensillum with sucrose plus W-7, a dose-dependent decrease of impulse frequency was observed when the concentration was <50 μM. In addition, when W-7 at 50 μM or higher concentration was added, an initial short-term impulse generation from the sugar receptor cell was observed, but this was followed by a silent period. When the sensillum was stimulated with W-7 plus a membrane-permeable cGMP analog, dibtyryl-cGMP or 8-bromo-cGMP, impulses of the sugar receptor cell were induced but the frequency was decreased. By the sidewall-recording method, we observed that the receptor potential induced by sucrose stimulation was decreased by W-7 in the sugar receptor cell, and corresponded with a disappearance of impulses. These data strongly suggest that the cGMP-gated channel generating receptor potential in the sugar receptor cell requires calmodulin for its gating.

Key words: CNG channel, contact chemoreceptor, electrophysiology, gustatory, insect, transduction

Introduction

The molecular mechanisms of signal transduction in the gustatory system are ambiguous. In the sugar receptor cell of flies, three different hypotheses have been proposed: (i) cyclic GMP (cGMP) acts as a second messenger to open cGMP-gated channels on the receptive membrane (Amakawa *et al.*, 1990); (ii) inositol 1,4,5-triphosphate (IP_3) acts as a second messenger (Koganezawa and Shimada, 2002); and (iii) the sugar receptor protein itself works as a channel, which is directly gated by sugar binding (Murakami and Kijima, 2000). Recently, Murata *et al.* (2004) reported that nitric oxide elicits a response in the sugar receptor cell in *Phormia regina* without any taste stimulation. This observation supports the hypothesis that the sugar receptor cell uses cGMP as a second messenger for its transduction mechanism, since nitric oxide is known as an activator of soluble guanylate cyclase. Our recent study suggested that the Gq protein is involved in adaptation rather than transduction in the sugar receptor cell (Seno *et al.*, 2005).

The contact-chemosensillum of flies is a hair-shaped organ housing five sensory neurons: four chemoreceptor neurons and one mechanoreceptor neuron (Ozaki and Tominaga, 1999). The chemoreceptor neurons are functionally differentiated to be deterrent, water, salt and sugar receptor cells, respectively (Dethier, 1976; Liscia and Solari, 2000; Ozaki *et al.*, 2003). However, they are morphologically similar to each other, having cell bodies, axons and dendritic processes as the receptive regions. The dendritic processes extending into the sensillum possess taste receptor proteins and all the functional molecules necessary for signal transduction (Morita, 1992).

In some sensory cells, calcium-binding proteins play a very important role as modulators of signal transduction. For

example, Ca^{2+}/calmodulin inhibits cyclic nucleotide-gated (CNG) channels and modifies adaptation levels in olfactory receptors (Chen and Yau, 1994) or photoreceptor cells (Hsu and Molday, 1993).

Previously, it has been reported that W-7, an inhibitor of calmodulin, suppressed the impulse of the sugar receptor cell in *Protophormia terraenovae* (Liscia *et al.*, 2002). However, the target of calmodulin was not clearly identified in their work. In this report, we attempted to certify the existence of calmodulin in the receptive region of the sugar receptor cell, and specify the target protein of calmodulin in *Phormia regina*.

Materials and methods

Flies

The blowflies (*P. regina*) used in this study were originally donated by Professor H. Morita (Kyusyu University). They were then reared in our laboratory by feeding on 0.1 M sucrose at 22 ± 2°C. Adult flies were used for electrophysiological experiments at 4–7 days after emergence.

Chemicals

All reagents were purchased from Wako Pure Chemical Industries, Ltd, except HEPES, EDTA and EGTA, which were purchased from Nakalai Tesque, Inc.

Purification of taste hairs

We cut the probosces of flies by hand and collected them in a dry plastic tube on ice. Using a previously reported freeze–vortex method (Ozaki *et al.*, 1993), we isolated the labellar chemosensilla by repeated freezing and vortexing, and the sensilla that adhered to the inner wall of the tube were collected.

Purification and identification of calcium binding proteins

Isolated sensilla were homogenized on ice and each washed three times with 0.4 ml of buffer A (10 mM Tris–HCl, pH 7.5, 1 mM EDTA, 0.2 mM PMSF). After every centrifugation (452 000 *g*, 1 h at 4°C) the supernatant was put into a tube and the same volume of buffer B (100 mM HEPES–HCl, pH 7.5, 4 mM $CaCl_2$, 200 mM NaCl) was added to the tube and centrifuged again. The supernatant was applied to a phenyl Sepharose column [bed volume 200 µl, phenyl Sepharose 6 Fast Flow (low sub), Amersham Biosciences Corp.], which was previously equilibrated by buffer C (50 mM HEPES–HCl, pH 7.5, 2 mM $CaCl_2$, 100 mM NaCl). The column was washed with 4 ml of buffer C and eluted with buffer D (50 mM HEPES–HCl, pH 7.5, 10 mM EGTA, 100 mM NaCl) to obtain 100 µl×1 and 20 µl×20 fractions. Proteins in fractions 2–21 were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) using a 12% acrylamide gel and stained by silver staining method. The main band was excised and analyzed by peptide mass fingerprinting after trypsin digestion.

Electrophysiological methods

For electrophysiological experiments, an isolated head of a fly was mounted under an optical microscope on a platinum indifferent electrode surrounded by a moist pad. Whole living flies were not used to avoid movement during response recording. The electrophysiological responses evoked from LL type chemosensilla, which were located on the outer margin of the labellum, were recorded through a computer-aided electrophysiological recording system (IDAC-USB, Syntech, Hilversum, The Netherlands). We used the tip-recording method (Hodgson *et al.*, 1955) for impulse recording from taste receptor cells, and the sidewall- recording method (Morita, 1959) for recording the receptor potential and the impulses at the same time. The ambient temperature was 20–24°C throughout the experiments. All stimulants were dissolved in 10 mM NaCl as an electrolyte.

When we used the tip-recording method, the LL type sensillar tip was stimulated with various stimulants in a recording glass capillary electrode for 15 s with a 10 min interval. Thus, all four of the functionally different chemoreceptor cells within the sensillum had the chance to respond, depending on the stimulus. Nevertheless, the receptor cell producing the recorded impulses was determined from the amplitude of the impulses, since each of the four receptor cells generates impulses of characteristic amplitudes. Impulse trains recorded from the sugar receptor cell consist of initial phasic and subsequent tonic parts. In the phasic period, for 150 ms after beginning of the stimulation, the impulse frequency shows a transient rapid increase that is not parallel with the receptor potential change. On the other hand, the impulse frequency in the tonic part gradually decreases in parallel with the receptor potential decrease following cell adaptation. In a time course of the magnitude of response, we counted the impulse numbers generated every 200 ms for 2 s only in the tonic part, because receptor potential only linearly reflects on impulse frequency in the tonic part but not in the phasic part. In this paper, therefore, we measured the cell sensitivity by counting impulse frequency in the tonic part, which was parallel with the receptor potential, and called the time course curve of impulse frequency in the tonic part the sensitivity curve.

When we used the sidewall-recording method, receptor potential of the exciting receptor cell was directly recorded as a DC component of the voltage change, which was induced by chemical stimulation. A recording glass capillary electrode was inserted into the outer lumen of a LL type of sensillum through its cuticle sidewall, and the sensillar tip was stimulated with various stimulants. Thus, the recorded potential was actually a reduced receptor potential, but we simply called it receptor potential according to Morita (1969).

Results

Purification and identification of calcium binding protein

We obtained 16.2 mg of isolated chemosensilla from 60 000 flies. Calcium binding protein candidates were eluted from the phenyl Sepharose column from fraction 5 (Figure 1). The apparent molecular mass of the most abundant protein was ~19 kDa. As a result of peptide mass finger printing, obtained mass data from nine peptides were identical with expected molecular masses of corresponding peptides derived from *Drosophila melanogaster* calmodulin (Table 1). This indicated that the most abundant calcium binding protein in chemosensilla of *P. regina* was calmodulin.

Effects of a calmodulin inhibitor on sugar receptor impulses caused by sucrose

We tested the effect of W-7 on the response of the sugar receptor cell in *P. regina* by the tip-recording method, in accordance with Liscia *et al.* (2002). When we stimulated chemosensilla with 100 mM sucrose plus 5, 10 or 20 μM of W-7, the obtained response was not obviously different from the response to 100 mM sucrose. When we used higher concentrations of W-7, so that the sensillum was stimulated with 100 mM sucrose plus 50, 100, 200 or 500 μM of W-7, we found an obvious silent period after an initial impulse train (Figure 2). As the concentrations of W-7 used increased from 50 to 500 μM, the duration of this impulse train became shorter (172 ± 19.3, 115 ± 17.2, 54.0 ± 6.6 and 37.7 ± 4.2 ms, mean ± SEM, n = 9) (Figure 3, open columns) and the subsequent silent period lasted longer (371 ± 129.6, 706 ± 88.2 and 1012 ± 127.2 ms, n = 9) (Figure 3, closed columns). Thus, the appearance of the silent period depending on W-7 concentration might suggest that W-7 can intensively suppress a specific period in a continuous impulse generation.

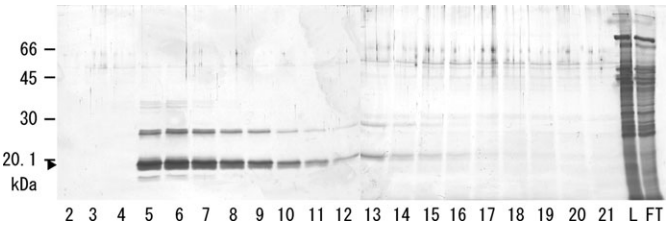

Figure 1 Calcium binding protein candidates obtained from isolated chemosensilla from *P. regina*. Twenty microliters of fractions 2–21 through a phenyl Sepharose column (see Materials and methods) was applied to SDS–PAGE, and detected by silver staining. Lane L indicates load; FT, flow through. Putative bands of calmodulin are indicated by an arrowhead.

Table 1 Peptide mass data of most abundant calcium binding protein in *P. regina* chemosensilla

Amino acid	Observed mass	Sequence[a]
2–14	1563.70	ADQLTEEQIAEFK (acetyl)
15–31	1843.93	EAFSLFDKDGDGTITTK
32–38	804.43	ELGTVM
79–91	1595.76	DTDSEEEIREAFR
92–107	1737.88	VFDKDGNGFISAAELR
96–107	1248.62	DGNGFISAAELR
108–116	1027.52	HVMTNLGEK
117–127	1348.64	LTDEEVDEMIR
117–127	1364.65	LTDEEVDEMIR (oxidation)
128–149	2478.10	EADIDGDGQVNYEEFVTMMTSK
128–149	2494.08	EADIDGDGQVNYEEFVTMMTSK (oxidation)

[a]Corresponding sequence in *D. melanogaster* calmodulin.

In the impulse train shown at the bottom of Figure 2, unusual impulse generation indicating irregular bursts was seen (Figure 2). Such an unusual impulse generation was induced by 100 mM sucrose plus 500 μM W-7 or sometimes by 100 mM sucrose plus 200 μM W-7 (data not shown). Those irregular bursts were supposed to be injury discharge caused by receptor cell membrane disturbance with high concentrations of W-7. When the receptor membranes of the four receptor cells in a sensillum were exposed to a high concentration of 500 μM W-7 at the same time, injury discharge would occur not only in the sugar receptor cell but also in other receptor cells. The smaller amplitude of the impulses was probably derived from the water receptor cell.

At low concentrations of W-7, we found no such intensive effects on the response of the sugar receptor cell. However, when comparing sensitivity curves in Figure 4 we noticed in the time courses of the sugar receptor cell activity that the gradual decrease in impulse frequency was accelerated in a W-7 concentration-dependent manner particularly at the lower concentration range (5–20 μM), though the impulse generation was overall depressed at higher concentrations (50–200 μM) (Figure 4). This might suggest that W-7 at lower and higher concentrations affects the sugar receptor cell in different ways.

Figure 2 shows the small amplitude impulses of the water receptor cell. However, the impulse generation of the water receptor cell was also inhibited by 100 or 200 μM W-7, so silent periods on the water receptor cell were also seen, although they were shorter than those on the sugar receptor cell. This observation on the water receptor was consistent with a previous report in *Protophormia terraenovae* (Liscia *et al.*, 2002).

W-5 is known as a low activity analog of W-7. When 100 μM W-5 was used instead of W-7, the impulse frequency of the sugar receptor cell was still reduced, but no silent period was seen (Figure 5A). Sensitivity curves in Figure 5B show that the inhibitory effect of W-7 was significantly larger than that of W-5 until ~2 s after the beginning of stimulation. Especially on appearance of the silent period after an

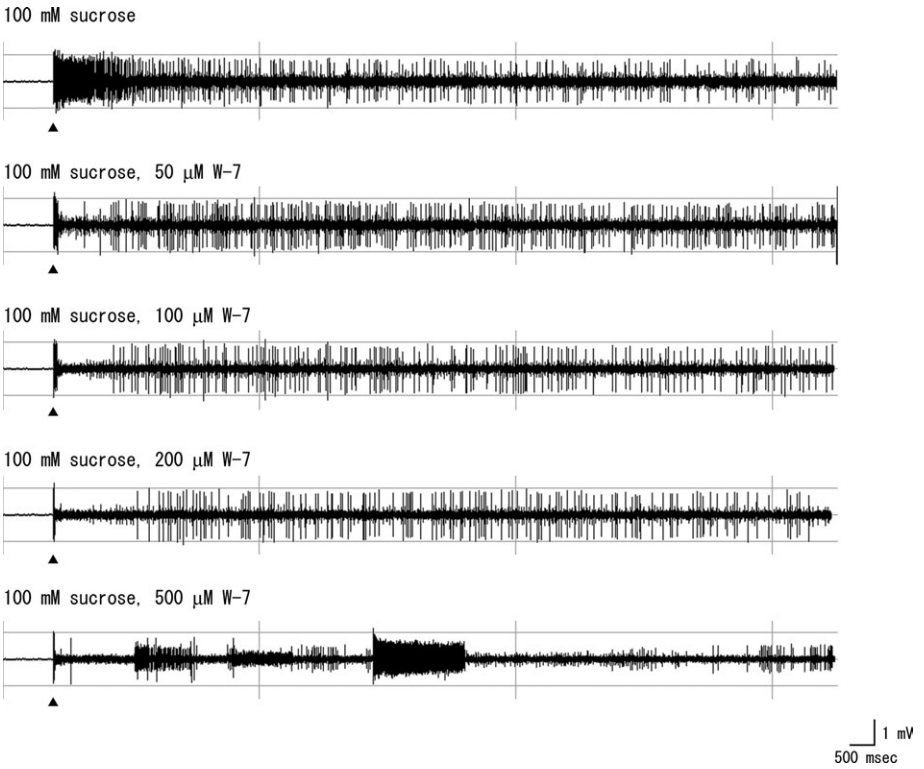

Figure 2 Representative responses of a chemosensillum stimulated with 100 mM sucrose containing various concentrations of W-7. Chemosensillum was stimulated with 100 mM sucrose containing 0, 50, 100, 200 or 500 μM W-7. Responses were recorded using a tip-recording method. Arrowheads indicate the beginning of stimulation.

initial impulse discharge, an effect of the calmodulin inhibitor W-7 was strongly suggested. Nevertheless, we could not exclude the possibility that W-7 affected the sugar receptor cell via another mechanism than inhibition of calmodulin.

Effect of a calmodulin inhibitor on sugar receptor impulses caused by membrane-permeable cGMP

It has been reported that stimulation of a chemosensilla with dibtyryl cyclic GMP (DBcGMP) or 8-bromo cyclic GMP (8BrcGMP), a membrane-permeable cGMP analog, causes sugar receptor cell activation (Amakawa *et al.*, 1992). It has been thought that DBcGMP or 8BrcGMP penetrates into the sugar receptor cell and opens the CNG channels on the receptive membrane of the sugar receptor cell, mimicking the intrinsic intracellular second messenger, cGMP.

These cGMP analogs elicited impulses of the sugar receptor cell, although the sensitivity curves to DBcGMP and 8BrcGMP were different from that to sucrose and from each other, as was previously reported (Amakawa *et al.*, 1992). However, the responses to both cGMP analogs were strongly inhibited by 100 μM W-7 (Figures 6 and 7). The number of impulses to 20 mM DBcGMP, which were generated during 200 ms at the beginning of the tonic response, were 13.8 ± 1.37 (n = 9) and were significantly decreased to 2.56 ± 0.818 (n = 9) in the presence of 100 μM W-7. The number of impulses to 20 mM 8BrcGMP, which were generated during 200 ms at the beginning of the tonic response, were 5.86 ± 0.508 (n = 9) and were significantly decreased to 1.86 ± 0.595 (n = 9) in the presence of 100 μM W-7. As has been suggested by Amakawa *et al.* (1992), if DBcGMP or 8BrcGMP directly gates the CNG channel from inside the cell, this result suggests that calmodulin enhanced the CNG channel opening by cGMP.

Effects of a calmodulin inhibitor on sugar receptor potential caused by sucrose

Hypothesizing that DBcGMP or 8BrcGMP gated the CNG channel to produce the receptor potential in the sugar receptor cell, we tried to understand our results obtained by the tip-recording method, and reached an explanation that calmodulin would be involved in the CNG channel opening. By the tip-recording method, however, we could only observe impulse discharge but not receptor potential. In most cases, the receptor potential of insect chemosensory cells, which was caused by gating ion channels, promoted impulse generation via voltage-gated channels. If such a voltage-gated channel could directly be inhibited by W-7, impulses would be reduced but receptor potential would not. Otherwise, if the ion channel for producing receptor potential could be inhibited by W-7, not only impulses but also receptor potential would be inhibited by W-7 at the same time. In order to

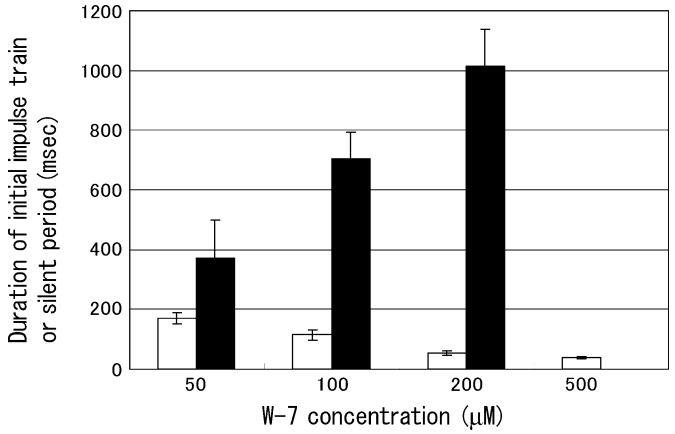

Figure 3 Dose dependency of the W-7 effect on the duration of the initial impulse train and the silent period. Durations of initial impulse trains appeared at the beginning of stimulation with 100 mM sucrose containing various concentrations of W-7, and are represented by open columns (n = 9). Durations of silent periods caused by 100 mM sucrose containing various W-7 concentrations are represented by closed columns (n = 9). Responses were recorded using a tip-recording method.

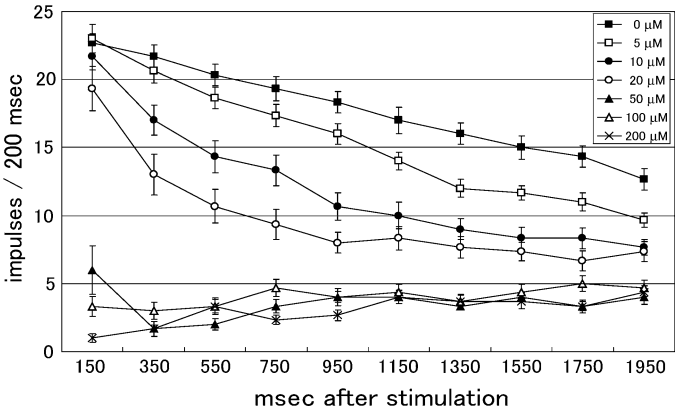

Figure 4 Effect of W-7 on the sensitivity curve of the sugar receptor cell. Chemosensilla were stimulated with 100 mM sucrose containing various concentrations of W-7. The impulse numbers generated every 200 ms are plotted until 2 s after the beginning of stimulation (mean ± SEM, n = 10). Responses were recorded using a tip-recording method.

confirm which was the case, we recorded the response to sucrose in the sugar receptor cell by the sidewall-recording method in the absence or the presence of W-7. When the chemosensillum was stimulated by 100 mM sucrose, receptor potential and impulse generation were observed at the same time during the stimulation. When it was stimulated with 100 mM sucrose plus 200 μM W-7, a short-term receptor potential and impulse train was followed by a silent period (Figure 8). As long as the receptor potential was observed, impulses were evoked regardless of the absence or presence of W-7, but they rapidly diminished in a continuous stimulation period in the presence of W-7. These data indicated that W-7 directly inhibited receptor potential generated at the receptive membrane and indirectly inhibited impulses produced at the impulse-generating site.

Effect of Ca^{2+} ion chelator in sensillar lymph on sugar receptor impulses

If the calcium influx through CNG channels enhanced calmodulin activity, resulting in positive feedback in the sugar taste receptor cell, we could mimic W-7 dependent inhibition of the response in the sugar receptor cell by decreasing the calcium concentration in the sensillar lymph surrounding the dendritic processes of taste cells. Because of this working hypothesis, we compared sugar receptor response to 100 mM sucrose in the absence and the presence of 10 mM BAPTA. At the beginning of the experiments, therefore, we expected that BAPTA would suppress the impulse generation to sucrose stimulation. As shown in Figure 9, BAPTA did affect the sugar receptor cell, but unexpectedly, the impulse generation was significantly increased. Thus, the decrease in extracellular calcium concentration activated the impulse

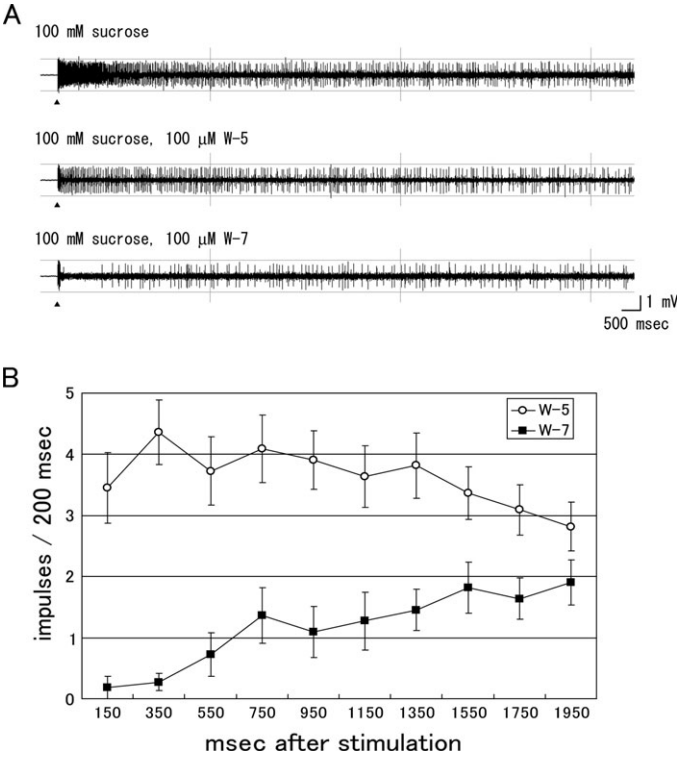

Figure 5 Different effects of W-7 and W-5 on sugar receptor cell response. Responses were recorded using a tip-recording method. **(A)** Representative response of a chemosensillum stimulated with 100 mM sucrose, 100 mM sucrose containing 100 μM W-5 or 100 mM sucrose containing 100 μM W-7. Arrow heads indicate the beginning of stimulation. **(B)** Sensitivity curves of sugar receptor cells stimulated with 100 mM sucrose containing 100 μM W-7 and W-5, respectively (mean ± SEM, n = 11). The impulse numbers generated every 200 ms are plotted until 2 s after the beginning of stimulation.

generation in the sugar receptor cell, so that the calcium required for calmodulin activation to enhance the CNG channel opening, which may be inhibited by W-7, did not come

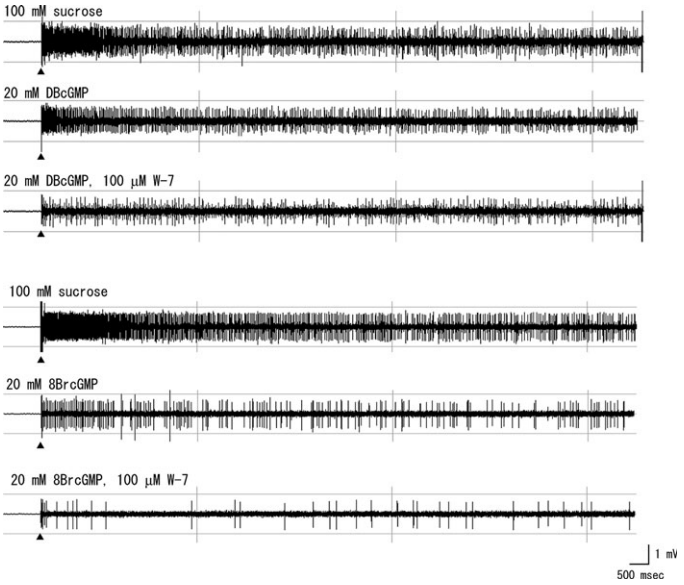

Figure 6 Effect of W-7 on sugar receptor cell response evoked by membrane-permeable cGMP analogs. Representative responses of two chemosensilla; one was stimulated with 100 mM sucrose, 20 mM DBcGMP or 20 mM DBcGMP containing 100 μM W-7, and the other was stimulated with 100 mM sucrose, 20 mM 8BrcGMP or 20 mM 8BrcGMP containing 100 μM W-7. Arrowheads indicate the beginning of stimulation. Responses were recorded using a tip-recording method.

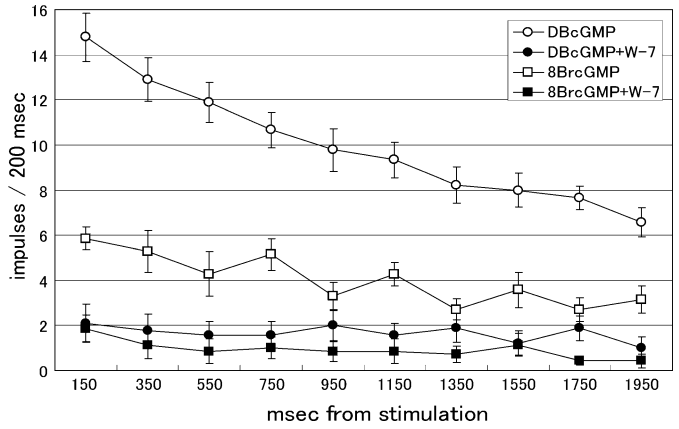

Figure 7 Effect of W-7 on the sensitivity curve of a sugar receptor cell stimulated with membrane-permeable cGMP analogs. The impulse numbers generated every 200 ms are plotted until 2 s after the beginning of stimulation with 20 mM DBcGMP (n = 9), 20 mM DBcGMP containing 100 μM W-7 (n = 9), 20 mM 8Bromo-cGMP (n = 7) or 20 mM 8BrcGMP containing 100 μM W-7 (n = 7) (mean ± SEM). Responses were recorded using a tip-recording method.

from the sensillar lymph. There may be a different explanation for activation of the sugar receptor cell responding to sucrose via chelating of extracellular calcium by BAPTA and via enhancement of CNG channel opening by the calcium/calmodulin system.

Discussion

Inhibitory effects of W-7 on the sugar receptor cell response

Using isolated chemosensillar preparations, we identified calmodulin as a major calcium binding protein that is localized in the dendritic processes of taste cells (Figure 1). It is therefore suggested that calmodulin has a function in the receptive region of taste cells. In the presence of ≤20 μM W-7, the sugar receptor cell activity was gradually suppressed (Figure 4). Liscia *et al.* (2002) have already reported that the calmodulin inhibitor, W-7, suppresses electrophysiological impulses of the sugar receptor cell in blowfly, *Protophormia terraenovae*. From the perspective of suppression in sugar receptor cell response, our results were consistent with their results, but we noticed further findings in *Phormia regina*. In particular, a characteristic short-term phasic impulse train and a following silent period in the presence of ≥50 μM W-7 in *P. regina* (Figures 2 and 3) have not been mentioned in *P. terraenovae* (Liscia *et al.*, 2002). These phenomena were not induced by W-5 (Figure 5), and considered the involvement of calmodulin in the sugar taste transduction mechanism of *P. regina*.

When we continuously stimulated chemosensilla with sucrose in the presence of ≥50 μM W-7, at the very beginning of stimulation the phasic impulse discharge of the sugar receptor cell started as usual but then diminished, followed by a silent period in which no impulses were observed (Figure 2). Thus, the duration of the phasic impulse discharge shortened and the silent period lasted in a W-7 concentration-dependent manner (Figure 3), and subsequently impulses of the sugar receptor cell reappeared.

We have two hypotheses to explain why the sugar receptor cell was insensitive to W-7 at the beginning of stimulation. There may be some lag time until W-7 affects the response to sucrose, probably because signal transduction of the sugar receptor cell starts before W-7 reaches its target. Alternatively, W-7 does not affect the phasic response, probably because calmodulin is not involved in the transduction mechanism in early phasic response generation.

It is known that an impulse train recorded from the fly sugar receptor cell consists of an initial phasic part and subsequent tonic parts. The phasic period, ~150 ms at the beginning of stimulation, is characterized with a transient increase in the impulse frequency, which is not parallel with the receptor potential. On the other hand, the impulse frequency in the tonic part decreases in parallel with the receptor potential, as cell adaptation gradually progresses (Ozaki and Amakawa, 1992). Although the mechanism for occurrence of the phasic and subsequent tonic responses are not clear, our results, showing retardation of the tonic response or appearance of silent period in the presence of W-7, might suggest that W-7 inhibits initiation of the tonic response. On the other hand, the apparently continuous

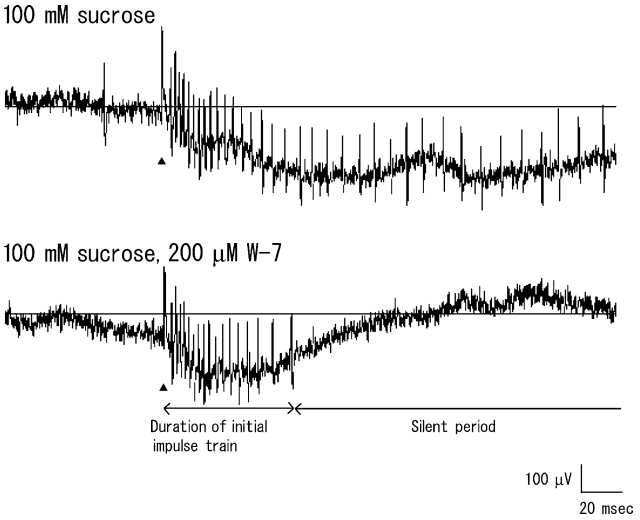

Figure 8 Effect of W-7 on receptor potential. A chemosensillum was stimulated with 100 mM sucrose (top) or 100 mM sucrose containing 200 μM W-7 (bottom), and the response was recorded by sidewall recording. Arrowheads indicate the beginning of stimulation.

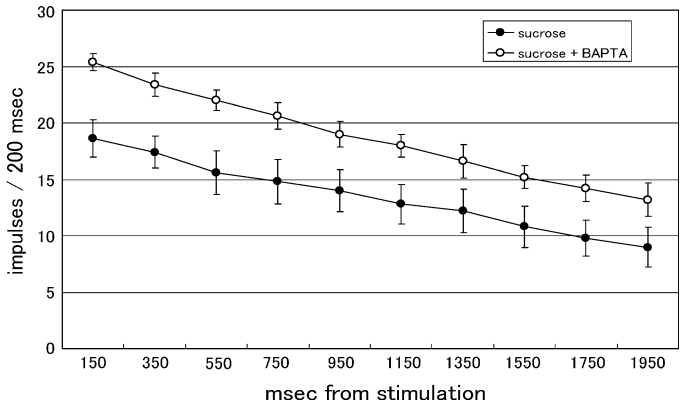

Figure 9 Effect of BAPTA on the sensitivity curve of the sugar receptor cell. A chemosensillum was stimulated with 100 mM sucrose or 100 mM sucrose containing 10 mM BAPTA. The impulse numbers generated every 200 ms are plotted until 2 s after the beginning of stimulation (mean ± SEM, n = 5). Responses were recorded using a tip-recording method.

tonic part might consist of two periods, which could be both W-7 sensitive and insensitive, and the W-7-sensitive period would selectively turn to the silent period in the presence of W-7. These three different phases in the chemosensillum response are remeniscent of the three different phases of the pheromone response in *Manduca sexta* olfactory receptor neurons (Kaissling, 1986).

Target point of calmodulin in the sugar taste transduction

One of the major hypotheses about signal transduction in the sugar receptor cell of *P. regina* is that cGMP acts as a second messenger to open CNG channels on the receptor membrane (Amakawa *et al.*, 1990). In accordance with this hypothesis, we could make a feasible explanation for our results; impulses of the sugar receptor cell, when stimulated with DBcGMP or 8BrcGMP, were suppressed by W-7 (Figures 2–7). Using the tip-recording method, we observed impulses to DBcGMP or 8BrcGMP. As discussed by Amakawa *et al.* (1990), the sugar receptor cell in *P. regina* has a nucleotide receptor site (N site), and DBcGMP can bind to this N site as an agonist but 8BrcGMP as an antagonist. Thus, the impulses induced by these cGMP analogs suggested that 8BrcGMP or a portion of DBcGMP could penetrate into the cell and directly open the CNG channel on the receptive membrane from the inside, mimicking intrinsic intracellular second messengers in the sugar receptor cell. If the opening of the CNG channel on dendritic processes gave a large enough magnitude of receptor potential to open the voltage-gated channel near the cell body, the impulse generation was triggered.

However, when we measured impulses by the tip-recording method we could not accurately identify the W-7-sensitive calmodulin-related step in the taste transduction and/or the impulse generation processes. Hence, by using the sidewall-recording method to detect the receptor potential, we confirmed that W-7 suppressed the receptor potential before the impulses (Figure 8) and suggested that the target of calmodulin is the CNG channel required for the receptor potential generation.

On the other hand, calmodulin is known as an activator of phosphodiesterase (PDE) (Means and Dedman, 1980). However, if calmodulin activated PDE in the sugar receptor cell and accelerated hydrolysis of cGMP, calmodulin inhibitors would suppress hydrolysis of cGMP, resulting in an increase of cGMP. This in turn would enhance impulse generation in the sugar receptor cell. However, this was not the case in our results. PDE may not be a target of calmodulin in the sugar receptor cell.

Previously, it was reported in olfactory receptor (Chen and Yau, 1994) or photoreceptor cells (Hsu and Molday, 1993) that Ca^{2+}/calmodulin inhibited CNG channels and that adaptation levels were modified. Our data also suggested that the function of taste receptor cells was modified by calmodulin, whose target is the CNG channel. However, interestingly in our case, Ca^{2+}/calmodulin seemed to be required for CNG channel opening. This was totally different from the effects of calmodulin in other sensory systems, in which Ca^{2+}/calmodulin inhibited CNG channel opening. There are few papers on calmodulin in invertebrate chemosensory systems, but *paramaecium* (Saimi and Kung, 1994) has an ion channel, which is activated by Ca^{2+}/calmodulin.

Where does the Ca^{2+} needed to activate calmodulin derive from? If the calcium influx induced by the CNG channel opening in the sugar taste receptor cell leads to activation of calmodulin, the sugar receptor cell has a positive feedback loop. However, extracellular application of the calcium chelator, BAPTA, did not mimic the effect of W-7 but increased the sensitivity of the cell to sucrose (Figure 9). Thus, we

thought that calcium, which is required for calmodulin activation, does not come from the sensillar lymph but from some intracellular storage, although there are no mitochondria or endoplasmic reticulum in the dendritic processes (Ozaki and Tominaga, 1999). Considering a previous report by Ozaki and Amakawa (1992), it can be presumed that BAPTA enhanced the response of the sugar receptor cell via inhibition of protein kinase C (PKC). Activation of PKC by Ca^{2+} and/or IP_3 was reported to accelerate the sugar receptor cell adaptation in *P. regina*.

There are still two possible hypotheses for signal transduction mechanisms without CNG channels in the fly sugar receptor cells: sugar-gated channels (Murakami and Kijima, 2000) or IP_3 dependent channels (Koganezawa and Shimada, 2002) are involved in receptor potential generation and they might be targets of calmodulin. However, we could not find appropriate explanations to fit these hypotheses, and we observed that the impulses of the sugar receptor cell were caused by membrane-permeable cGMP analogs and inhibited by W-7. One potent explanation is that for a continuous receptor potential generation, different signal transduction mechanisms regulating different channels work in parallel and/or sequentially. Calmodulin might act on one of them. Considering the effects of W-7 on an impulse train to sucrose stimulation, we observed three different parts of the impulse train: the initial phasic impulses, the subsequent W-7-sensitive impulses and the W-7-insensitive tonic impulses. Because only the impulses following the initial phasic part were completely depressed by a calmodulin inhibitor, CNG channel opening might mainly induce this type of impulse. Impulses in the initial phasic part might be induced by another type of channel opened directly by sugar binding, as proposed by Murakami and Kijima (2000). Moreover, we could not exclude other possibilities, e.g. that W-7 acts as an unspecific inhibitor of calcium-activated nonselective (CAN) cation channels. BAPTA might prevent the calcium-dependent blocking of other calcium-permeable transduction channels, which are active before the CAN channels in the previous phases of the sugar response. Future investigations are needed to elucidate the molecular mechanism of calmodulin involvement, especially for channel opening.

Acknowledgements

This study was supported by a grant from ProBRAIN to M.O.

References

Amakawa, T., Ozaki, M. and **Kawata, K.** (1990) *Effects of cyclic GMP on the sugar taste receptor cell of the fly* Phormia regina. J. Insect Physiol., 36, 281–286.

Amakawa, T., Kawata, K. and **Ozaki, M.** (1992) *Nucleotide receptor-site on the labellar sugar receptor cell of the blowfly* Phormia regina. J. Insect Physiol., 38, 365–371.

Chen, T.Y. and **Yau, K.W.** (1994) *Direct modulation by Ca^{2+}-calmodulin of cyclic nucleotide-activated channel of rat olfactory receptor neurons*. Nature, 368, 545–548.

Dethier, V.G. (1976) The Hungry Fly. Harvard University Press, Cambridge, MA.

Hodgson, E.S., Lettvin, J.Y. and **Roedar, K.D.** (1955) *Physiology of a primary chemoreceptor unit*. Science, 122, 417–418.

Hsu, Y.T. and **Molday, R.S.** (1993) *Modulation of the cGMP-gated channel of rod photoreceptor cells by calmodulin*. Nature, 361, 76–79.

Kaissling, K.S. (1986) *Chemo-electrical transduction in insect olfactory receptors*. Annu. Rev. Neurosci., 9, 121–145.

Koganezawa, M. and **Shimada, I.** (2002) *Inositol 1,4,5-trisphosphate transduction cascade in taste reception of the fleshfly,* Boettcherisca peregrina. J. Neurobiol., 51, 66–83.

Liscia, A. and **Solari, P.** (2000) *Bitter taste recognition in the blowfly: electrophysiological and behavioral evidence*. Physiol. Behav., 70, 61–65.

Liscia, A., Crnjar, R., Masala, C., Sollai, G. and **Solari, P.** (2002) *Sugar reception in the blowfly: a possible Ca++ involvement*. J. Insect Physiol., 48, 693–699.

Means, A. and **Dedman, J.** (1980) *Calmodulin—an intracellular calcium receptor.* Nature, 285, 73–77.

Morita, H. (1959) *Initiation of spike potentials in contact chemosensory hairs of insects. III. D.C. stimulation and generator potential of labellar chemoreceptor of* Calliphora. J. Cellular Comp. Physiol., 54, 189–204.

Morita, H. (1969) *Electric signs of taste receptor activity.* In Pfaffmann, C. (ed.), Olfaction and Taste III. Pergamon Press, Oxford, pp. 370–381.

Morita, H. (1992) *Transduction process and impulse initiation in insect contact chemoreceptor.* Zoological Sci., 9, 1–16.

Murakami, M. and **Kijima, H.** (2000) *Transduction ion channels directly gated by sugars on the insect taste cell*. J. Gen. Physiol., 115, 455–466.

Murata, Y., Mashiko, M., Ozaki, M., Amakawa, T. and **Nakamura, T.** (2004) *Intrinsic nitric oxide regulates the taste response of the sugar receptor cell in the blowfly,* Phormia regina. Chem. Senses, 29, 75–81.

Ozaki, M. and **Amakawa, T.** (1992) *Adaptation-promoting effect of IP_3, Ca^{2+}, and phorbol ester on the sugar taste receptor cell of the blowfly,* Phormia regina. J. Gen. Physiol., 100, 867–879.

Ozaki, M. and **Tominaga, Y.** (1999) *Contact Chemoreceptors*. In Eguchi, E. and Tominaga, Y. (eds), Atlas of Arthropod Sensory Receptors, Dynamic Morphology in Relation to Function. Springer-Verlag, Tokyo, pp. 143–154.

Ozaki, M., Amakawa, T., Ozaki, K. and **Tokunaga F.** (1993) *Two types of sugar-binding protein in the labellum of the fly.* J. Gen. Physiol., 102, 201–216.

Ozaki, M., Takahara, T., Kawahara, Y., Wada-Katsumata, A., Seno, K., Amakawa, T., Yamaoka, R. and **Nakamura, T.** (2003) *Perception of noxious compounds by contact chemoreceptors of the blowfly,* Phormia regina*: putative role of an odorant-binding protein*. Chem. Senses, 28, 349–359.

Saimi, Y. and **Kung, C.** (1994) *Ion channel regulation by calmodulin binding*. FEBS Lett., 350, 155–158.

Seno, K., Fujikawa, K., Nakamura, T. and **Ozaki, M.** (2005) *$G_{q\alpha}$ subunit mediates receptor site-specific adaptation in the sugar taste receptor cell of the blowfly,* Phormia regina. Neurosci. Lett., 377, 200–205.

Accepted May 11, 2005